Release Notes from v1.1 to v1.2: Typo corrected, "Variants generating PTCs **5'** of c.1714 of NM_000545.8..." corrected to "Variants generating PTCs **3'** of c.1714 of NM_000545.8..."

Gene	Disease (MONDO ID)	Transcript
HNF1A	Monogenic Diabetes (MONDO:0015967)	NM_000545.8

PATHOGENIC CRITERIA			
	Rule	Original Description	Specifications
VS	PVS1	Null variant in a gene where LOF is a known mechanism of disease	Use *HNF1A* PVS1 decision tree.
	PS2_Very Strong	*De novo* in patient with disease	Use SVI recommended point-based system with specifications for "Phenotype Consistency" described below.
Strong	PVS1_Strong	Null variant in a gene where LOF is a known mechanism of disease	Use *HNF1A* PVS1 decision tree.
	PS1	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change	No change.
	PS2	*De novo* in patient with disease	Use SVI recommended point-based system with specifications for "Phenotype Consistency" described below.
	PS3	Well-established *in vitro* or *in vivo*, functional studies supportive of a damaging effect on the gene or gene product	Applicable to non-canonical splice site variants that have RNA and in silico evidence of aberrant splicing.
	PS4	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	Seven or more= Strong. Variant should meet PM2_Supporting in order to use PS4 at any level (careful review of gnomAD QC data may be necessary to assess whether variant is real or an artifact, especially if variant is in a polyC region). Phenotype of affected individuals must include diabetes, without clear evidence of an autoimmune etiology (see below).
	PP1_Strong	Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease	Use thresholds suggested by Jarvik and Browning (PMID: 27236918) Single Family >1 Family Strong ≤ 1/32 (5 meioses) ≤ 1/16 (4 meioses)

	PM5_Strong	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before	Applicable once two amino acid changes have been classified as pathogenic at the same amino acid residue
Moderate	PM1	Located in a mutational hot spot and/or critical and well-established functional domain without benign variation	This criterion can be used for variants in residues that directly bind DNA (see below).
	PM3	For recessive disorders, detected in trans with a pathogenic variant	Not applicable
	PM4	Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants	For single amino acid deletions, use as supporting level of evidence.
	PM5	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before	The novel amino acid change must have a Grantham distance greater than or equal to the previously classified pathogenic variant.
	PS2_Moderate (formerly PM6)	*De novo* in patient with disease	Use SVI recommended point-based system with specifications for "Phenotype Consistency" described below.
	PP1_Moderate	Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease	Use thresholds suggested by Jarvik and Browning (PMID: 27236918) Single Family >1 Family Moderate ≤ 1/16 (4 meioses) ≤ 1/8 (3 meioses)
	PM1_Supporting	Located in a mutational hot spot and/or critical and well-established functional domain	Use for defined regions in the DNA binding and dimerization domains. It can also be used for variants within certain transcription factor binding sites of the promoter.
	PS4_Moderate	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	4-6 unrelated occurrences = Moderate. Variant should meet PM2_Supporting in order to use PS4 at any level. Phenotype of affected individuals must include diabetes, without clear evidence of an autoimmune etiology (see below).

	PS3_Moderate	Well-established *in vitro* or *in vivo*, functional studies supportive of a damaging effect on the gene or gene product	Applicable for variants with luciferase assay data (evidence of decreased transactivation (<=40% of wild type) by the Gloyn/Oxford group (Althari et al (2020) (DOI: https://doi.org/10.1016/j.ajhg.2020.08.016).
	PP4_Moderate	Patient's phenotype or family history is highly specific for a disease with a single genetic etiology	MODY Probability Calculator (MPC) result ≥50% chance of testing positive https://www.diabetesgenes.org/mody-probability-calculator/) AND negative *HNF4A* testing AND presence of at least one additional feature characteristic of HNF1A-MODY: -Antibody negative and/or persistent C-peptide after five years post- T1DM diagnosis -Response to low-dose SU (extreme response-hypoglycemia) -Low hsCRP in patient with clinical diagnosis of T2DM -Biochemical/Molecular phenotypic evidence from patient cell lines -Hepatocellular adenomas
Supporting	PP1	Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease	Use thresholds suggested by Jarvik and Browning (PMID: 27236918) Supporting: Single Family ≤ 1/8 (3 meioses); >1 Family ≤ ¼ (2 meioses)
	PP2	Missense variant in gene with low rate of benign missense variation and where missense variants are a common mechanism of disease	Not applicable.
	PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product	Use REVEL score of ≥0.70 as supportive evidence of pathogenicity. We also support using SpliceAI to assess the predicted impact of non-canonical splicing variants and synonymous variants: apply PP3 when the predicted change is above 0.2 (Wai et al. 2020 PMID: 32123317; Jaganathan et al. 2019 PMID: 30661751).
	PP4	Patient's phenotype or family history is highly specific for a disease with a single genetic etiology	MODY Probability Calculator (MPC) result ≥50% chance of testing positive https://www.diabetesgenes.org/mody-probability-calculator/) AND negative *HNF4A* testing
	PP5	Reputable source reports as pathogenic	Not applicable

	PS1_ Supporting	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change	PS1 may also be used at a supporting level for canonical and non-canonical splicing variants when a different variant at the same nucleotide has been previously classified as pathogenic and the variant being assessed is predicted by SpliceAI to have a similar (SpliceAI score within 10% of the original variant) or greater deleterious impact.
	PS2_ Supporting	*De novo* in patient with disease	Use SVI recommended point-based system with specifications for "Phenotype Consistency" described below.
	PS3_ Supporting	Well-established *in vitro* or *in vivo*, functional studies supportive of a damaging effect on the gene or gene product	See list of approved functional studies and guidelines for interpretation of data (below).
	PM2_ Supporting	Absent in population databases	Prevalence ≤ 1:50,000 (≤ 0.00002 or 0.002%) in gnomAD European Non-Finnish population AND ≤ 1 copy in other founder and non-founder populations (would require ≤ 1/50,000 in non-European populations if/when they surpass 100,000 alleles).
	PM4_ Supporting	Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants	For single amino acid deletions/insertions, use as supporting level of evidence.
	PM5_ Supporting	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before	Apply if the previously classified amino acid change is likely pathogenic (rather than pathogenic) or if the previously classified variant is pathogenic but has a greater Grantham distance.
	PVS1_ Supporting	Null variant in a gene where LOF is aknown mechanism of disease	Use *HNF1A* PVS1 decision tree.

Probands (and/or family members when assessing segregation for PP1) with evidence of an autoimmune etiology of diabetes and/or absolute or near-absolute insulin deficiency will be excluded when assessing criteria that includes phenotype information. Such evidence includes the following:

- One or more positive diabetes autoantibodies (IA-2A, ZnT8A+, GAD) (PMIDs 21395678, 28701371, 30409810, 31704690)

- Very low or negative C-peptide, defined as either fasting or non-fasting random C-peptide (<200pmol/L or 0.6ng/mL) (PMID: 30225972, 23771925) or urinary C-peptide/creatinine ratio <0.2 nmol/mmol (PMID: 28701371 & 30409810)

An individual is considered "unaffected" if over age 70 and non-diabetic (based on Exeter work in PMID: 29026101 which shows penetrance of HNF1A-MODY at 98% by age 70).

Per guidance from ClinGen/SVI, one Supporting + 1 Very Strong piece of evidence is sufficient to classify a variant likely pathogenic

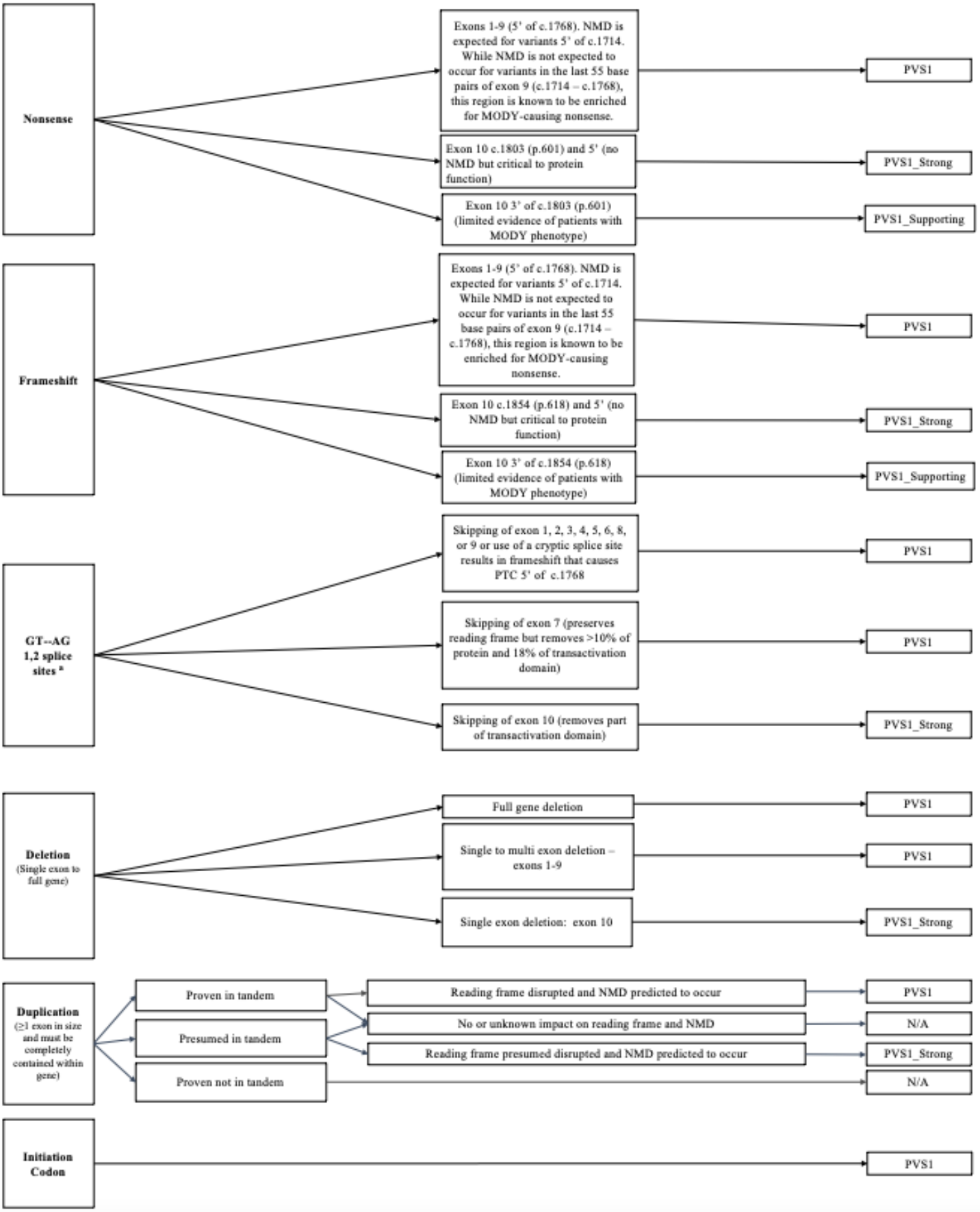
PVS1

- See below for adapted flowchart from ClinGen SVI group (Abou Tayoun, et al. 2018)
- Variants generating PTCs 3' of c.1714 of NM_000545.8, which includes the last 55 nucleotides of exon 9 and all of exon 10, are not expected to cause NMD (PMID: 24274751). The transactivation domain (TAD) of the protein overlaps with this region. The last 55 nucleotides of exon 9 (c.1714-1768) is enriched for disease-causing variants and loss-of function variants in this region have been found in patients/families with a MODY phenotype. Therefore, a "very strong" level of evidence will be used for loss-of-function variants 5' of c.1768 regardless of where the premature termination codon occurs.
    - PVS1_Strong will be applied to nonsense variants at c.1803 (p.601) and 5' and frameshift variants at c.1854 (p.618) and 5'. The distinction of nonsense and frameshift variants was made following a careful review of the phenotypes of individuals with loss-of-function variants in exon 10, which lead to our prediction that the addition of new amino acids from a frameshift will disrupt the TAD and cause a MODY phenotype more so than the deletion of a small part of the end of the TAD. Moderate phenotypic evidence was applied to the c.1802del (p.601Ter) variant, but the individual with the next nonsense variant (p.Gln625Ter) was unaffected. Frameshift variants at p.Ile618 and 5' have been identified in patients with a phenotype consistent with MODY.
- "Exon skipping or use of a cryptic splice site that preserves reading frame" and "Single to multi-exon deletion that preserves reading frame"
    - Deletions of exon 1 would lead at least to loss of the initiation codon (see below for recommendations for initiation codon variants). Deletions of single exons 2, 3, 4, 5, 6, 8 or 9 all cause frameshift, and thus PVS1 would be used. In *HNF1A*, only exon 7 (LRG_522t1) is surrounded by introns of the same phase. Skipping or deletion of exon 7 would remove 64 amino acids in the TAD, which is >10% of the protein and 18% of the TAD. Given the significance of the TAD, we support still using PVS1 instead of PVS1_Strong in this situation. A deletion of exon 10 would remove part of the TAD but less than 10% of the protein. Since the TAD is critical to protein function, and variants that disrupt all of exon 10 have been found in patients with a MODY phenotype, we will use PVS1_Strong for deletions of exon 10 and splicing variants that would predict the skipping of exon 10. This specification is in accordance with Tayoun's recommendation to use PVS1_Strong in cases in which the truncated region is critical to protein function.
- Apply PVS1 to initiation codon variants. Four initiation codon variants have been identified in patients with a MODY phenotype. The closest potential in-frame start codon is p.Met118. Starting the protein at

p.Met118 would remove 18% of the protein, including the entire dimerization domain. There are many P/LP variants upstream of p.Met118.

- Per recommendations from the SVI, when RNA analysis demonstrates abnormal splicing from non-canonical splice site variants, apply PS3 instead of PVS1.

ClinGen Monogenic Diabetes Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1.2
This version specified for the following genes: *HNF1A*
Expert Panel Page: https://www.clinicalgenome.org/affiliation/50016



PS2 (de novo evidence)

- Use SVI point-based system (see tables). This evidence can be used for the current patient, previous patient or a patient previously reported in the literature.
- To obtain maximum points ("phenotype highly specific for gene") patient must meet criteria for PP4 (result of ≥50% chance or higher of testing positive for MODY on the MODY Probability calculator (https://www.diabetesgenes.org/mody-probability-calculator/) AND have negative *HNF4A* testing). If patient does not meet PP4 but is noted to have diabetes, use points corresponding to "phenotype consistent with gene but not highly specific". If patient shows evidence of an autoimmune etiology for their diabetes and/or absolute or near-absolute insulin deficiency (see above), do not apply PS2.
- References
    - McDonald et al (2011) (PMID: 21395678)
    - Shields et al (2017) (PMID: 28701371)
    - Patel et al (2019) (PMID: 30409810)

Phenotypic consistency	Points per Proband	
	Confirmed de novo	Assumed de novo
Phenotype highly specific for gene	2	1
Phenotype consistent with gene but not highly specific	1	0.5

Table 2. Recommendation for determining the appropriate ACMG/AMP evidence strength level for de novo occurrence(s)

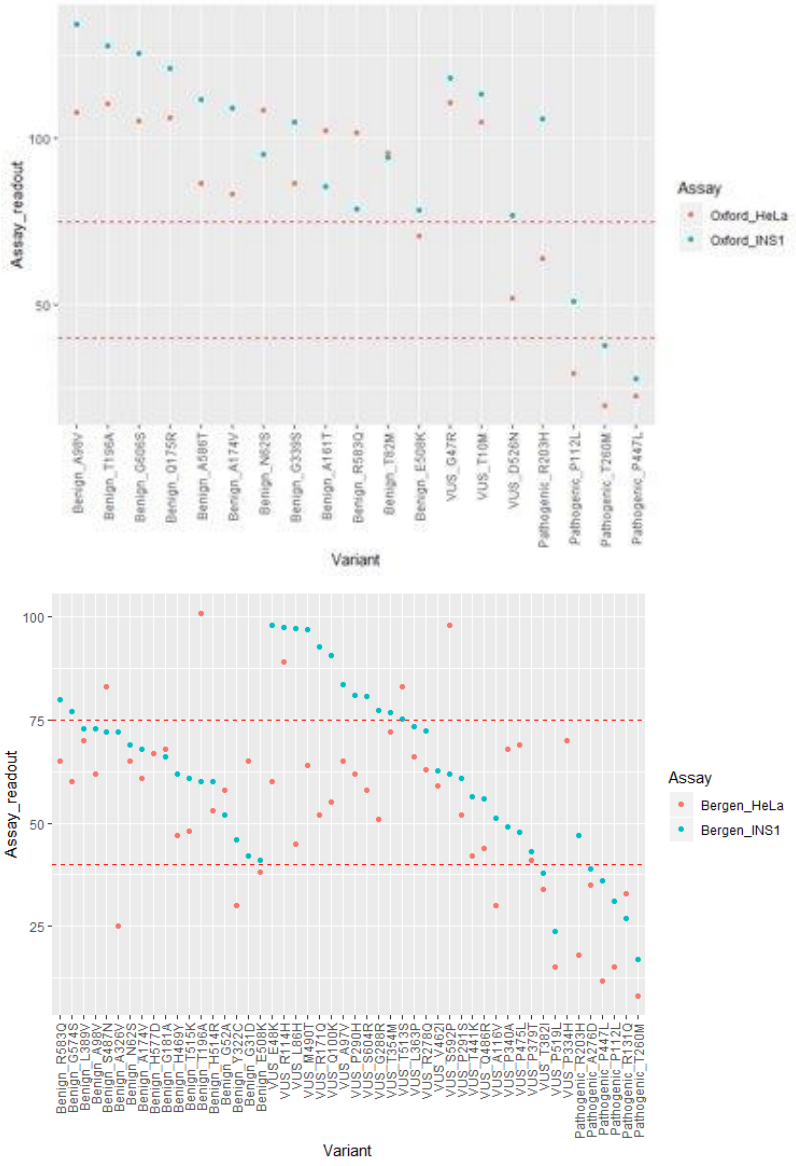
Supporting (PS2_Supporting or PM6_Supporting)	Moderate (PS2_Moderate or PM6)	Strong (PS2 or PM6_Strong)	Very Strong (PS2_VeryStrong or PM6_VeryStrong)
0.5	1	2	4

PS3_Suppporting

- **Luciferase assays for transactivation**
    - "Decreased function" is defined as activity less than 40% of wildtype (WT).
    - Note: this threshold is not 100% specific for transactivation (TA) activity and is complicated by the fact that TA activity will vary depending on many factors, for instance cell line (HeLa, INS, MIN6, etc.) and reporter construct that is used. Commonly used promoters include rat/human albumin (PMID:12574234) and HNF4A-P2 (PMID: 32910913). Experiments should be designed to include more than one promoter and more than one cell line; any data less than 40% of WT warrants use of PS3_Supporting or PS3_Moderate, with more weight given to assays that have appropriate controls (see below).
    - Assays should include controls for WT (human *HNF1A* cDNA (NM_000545.8)), T2DM-risk and known MODY variants, which represent the range of behaviours across the *HNF1A* allelic spectrum. When calculating an OddsPath or counting the total number of control variants per Brnich et al. 2020 (PMID: 31892348) the following are well-characterized pathogenic *HNF1A*-MODY variants: c.335C>T (p.Pro112Leu), c.779C>A (p.Thr260Met), c.1340C>T (p.Pro447Leu), c.1556C>T (p.Pro519Leu), c.787C>T p.(Arg263Cys) and c.686G>A

p.(Arg229Gln). We also recommend that assays include the following variants to capture dysfunction which increases risk for T2D but is insufficient to cause monogenic diabetes: c.293C>T (p.Ala98Val) and c.1522G>A (p.Glu508Lys) (PMID: 24915262, 26551672). The degree of TA impairment of *HNF1A* should correlate positively with the severity of the diabetes phenotype: disease-causing *HNF1A* variants impair TA activity more severely (<30-40% compared to WT) than T2D risk variants (40-75% compared to WT).

- We note that the data presented by the Oxford/Gloyn group in Althari et al (PMID:32910913) have four controls that can be classified as pathogenic based on non-functional data and 10 controls that can be classified as benign based on non-functional data. Using either HeLa or INS1 cells and cutoffs of 0.40/0.75, only ≤2 variants fall outside the expected range, and OddsPath based on the recommendations by Brnich et al 2020 (PMID: 31892348) can be achieved (>4.3) that enable the use of PS3_Moderate and BS3_Supporting. The data presented by the Bergen group in Althari et al (PMID: 32910913) used six controls that can be classified as pathogenic based on non-functional data and 18 controls that can be classified as benign based on non-functional data. Using either HeLa or INS1 cells and cutoffs of 0.40/0.75, the pathogenic variants in the Bergen data consistently fell into the expected TA range (<40%). However, two benign variants (not including T2D-risk variant p.Glu508Lys) fell into the pathogenic range and 14 out of 16 of the benign variants fell into the indeterminate range. We hence recommend applying PS3_Supporting and BS3_Supporting only at this time to data from this group.
- Validation of luciferase assay performed by Bergen and Oxford groups reported in Althari et al, with cutoffs of <40% of WT for functionally abnormal and ≥75% of WT for functionally normal:



- **EMSA for DNA binding**
  - "Decreased function" is defined as activity less than 40% of WT.
  - Note: the effect of the variant on DNA binding will be highly dependent on whether the variant is located within the DNA binding domain. Our experience is that for *HNF1A*-MODY causal variants located in the DNA binding domain, we observe DNA binding from 0-35% compared to WT. The following variants can be used as positive controls for reduced DNA binding ability (<40%): c.335C>T (p.Pro112Leu), c.608G>A (p.Arg203His), c.787C>T (p.Arg263Cys) and c.686G>A (p.Arg229Gln) (PMID: 11162430, 12574234, 24915262); we recommend the assay contain at least two. We also recommend that the assay include variants whose DNA binding ability has previously been assessed (e.g., c.298C>A (p.Gln100Lys) (~70% of WT) and c.392G>A (p.Arg131Gln) (~58% of WT) (PMID: 27899486).

- **Western blotting and indirect immunofluorescence for protein expression & localization**
    - Determining appropriate thresholds for protein expression is more difficult due to variability in results between different experimental protocols. Sample preparations, gel loading, transfer efficiency, antibody specificity, choice of internal control and quantification method are some of the factors that can contribute to varying and inconsistent results between labs. Altered protein expression can be indirectly captured through the read-out from a transactivation assay and reduced protein expression can provide an explanation for reduced transactivation.
    - When exploring protein mis-localisation we recommend that the c.589_615del (p.Lys197_Lys205del) variant is included as a positive control for impaired nuclear localization (cytosolic retention) (PMID: 16274290).
- Studies performed on a cell line generated from a patient sample (which will be heterozygous and also contain other variants in the patient's genome which could modify function) will not count as PS3 but instead will count toward PP4_Moderate.
- For canonical splice site variants, do not use PS3 for RNA studies demonstrating abnormal splicing, since PVS1 will already be used at some level. Per SVI recommendations, we will apply PS3 at the Strong level to non-canonical splice site variants that have RNA and in silico evidence of aberrant splicing.
- References
    - Najmi et al (2017) (PMID: 27899486)
    - Bjorkhaug et al (2003) (PMID: 12574234)
    - Brnich et al (2020) (PMID: 31892348)
    - Althari et al (2020) (PMID: 32910913)

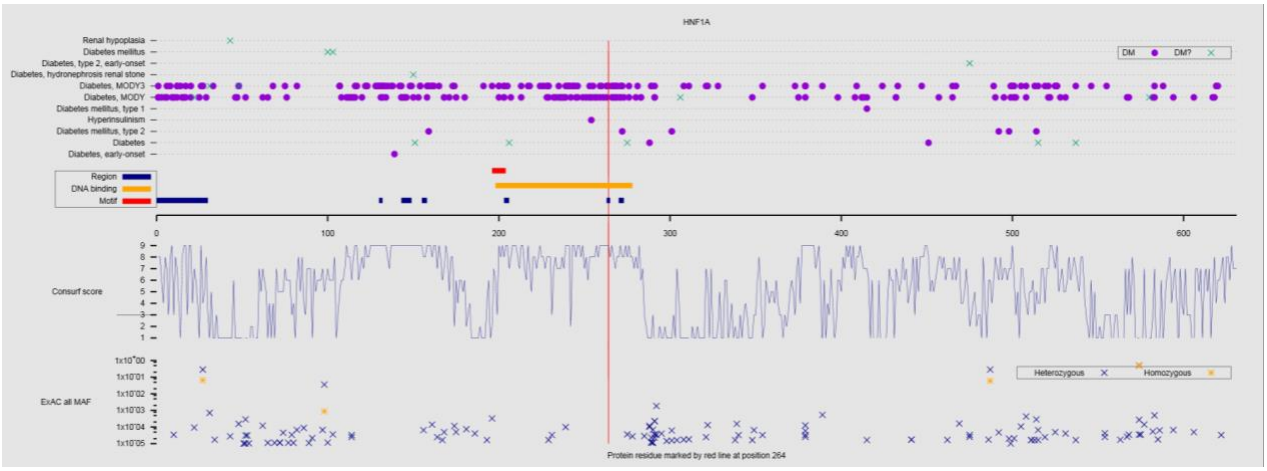
PS4

- Prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls.
    - 4-6 unrelated occurrences = Moderate, 7 or more = Strong
- Phenotype of the affected individual must include diabetes, with evidence of an autoimmune etiology and/or absolute or near-absolute insulin deficiency considered exclusionary (see above).
- Variant should meet PM2_Supporting in order to use PS4 at any level (careful review of gnomAD QC data may be necessary to assess whether variant is real or an artifact, especially if variant is in a polyC region).
- References
    - McDonald et al (2011) (PMID: 21395678)
    - Shields et al (2017) (PMID: 28701371)
    - Patel et al (2019) (PMID: 30409810)

PM1 and PM1_Supporting

- PM1: Applicable to amino acids that directly bind DNA (PMID: 12453420)
    - Gln130, Arg131, Glu132, His143, Leu144, Ser145, Gln146, His147, Leu148, Asn149, Lys155, Thr156, Gln157, Lys158, Arg203, Phe204, Lys205, Trp206, Arg263, Val264, Tyr265, Asn270, Arg271, Arg272, Lys273

- PM1_Supporting: Applicable for SNV and non-frameshift indel variants in the promoter, DNA binding and dimerization domains (PMID: 18003757)
    - Promoter
        - –c.-187 to c.-195 (AP1 binding site)
        - –c.-209 to c.-227 (Overlapping HNF3 & NF-Y sites)
        - –c.-238 to c.-259 (HNF1A binding site)
        - –c.-276 to c.-288 (HNF4A binding site)
    - Dimerization: codons 1-32, NM_000545.8
    - Subset of DNA binding domains: codons 107-174 and 201-280, NM_000545.8
- Plot showing the enrichment of pathogenic missense variants in the DNA binding and absence of common missense variants in gnomAD in this region:



- Note that although occurrence in the transactivation domain (codons 281-631, NM_000545.8 has been cited in older publications as evidence for causality, it is known that the transactivation domain is more tolerant to benign missense variation and therefore we will not apply PM1 at any level to variants within this region at this time (PMID: 11272211, 18003757, 23348805).

PP1

- We support using the thresholds recommended by Jarvik and Browning (PMID: 27236918)
- Variable penetrance and phenocopies complicate co-segregation studies. The presence of type 1 and type 2 phenocopies and significance of variants in unaffected individuals as defined above will need to be considered. If a family member(s) shows evidence of an autoimmune etiology for their diabetes and/or absolute or near-absolute insulin deficiency (see above), do not include them in PP1 calculation. See also discussion of unaffected individuals above.

PM2_Supporting

- Recommend using as supporting level of evidence (PM2_Supporting) per ClinGen guidance.
- Per guidance from ClinGen/SVI, PM2_Supporting + PVS1 is sufficient evidence of a variant being likely pathogenic
- We recommend investigating the genotype metrics in gnomAD for variants that have been flagged for having failed one or more quality parameters, as it is possible that some of these filtered variants are actually real. The number of filtered alleles can be counted to determine whether PM2_Supporting would be met even if they were genuine calls. Do not apply criterion if the filtered calls are sufficient in

number to not meet PM2_Supporting. Because it is also possible that these calls are false positives, do not use filtered variants to support BA1 or BS1.

PP2

- Missense variants account for 55% of all published pathogenic variants in this gene (Colclough et al 2013), however the constraint score for *HNF1A* (gene) is 1.07, which is not significant; therefore, we do not support using this criterion at this time. The low constraint score is most likely due to high tolerance for missense variants in the transactivation domain (see PM1 section). There are significantly more pathogenic missense variants in the DNA binding and dimerization domains, which are much less tolerant to missense variation. We may update this in the future if we can generate domain-specific scores.

PP4

- Patient should have a MODY Probability Calculator (MPC) result of ≥50% chance of testing positive (https://www.diabetesgenes.org/mody-probability-calculator/ PMID: 22218698). Given the similarities in phenotypes between *HNF1A*-MODY and *HNF4A*-MODY, the patient must also have negative *HNF4A* genetic analysis. Clinical judgement may need to be used when applying this criterion, as the MODY Probability Calculator is less reliable for non-Caucasians or people diagnosed >35.
- Certain assumptions can be made in order to use the MODY probability calculator:
    - Specific clinical information about parents not given but lab/literature states "Family history of diabetes"
        - Can click "Parent with diabetes" in calculator.
        - If no information about family history of diabetes is provided, attempt to use the calculator using both possibilities (yes/no). If this makes a difference in the ability to meet the PP4 cutoff (>50%), PP4 cannot be used.
    - Weight/Height/BMI not given but lab/literature states patient is "lean".
        - Enter BMI of 30.
    - HbA1c is not provided.
        - Attempt to use the calculator using values of 6% and 10%. If this makes a difference in the ability to meet the PP4 cutoff (>50%), PP4 cannot be used.
    - Treatment information is not provided.
        - Cannot use calculator.
- Presence of at least one of following features can elevate to PP4_Moderate
    - Antibody negative and/or persistent C-peptide after five years following T1DM diagnosis
    - Response to low-dose SU (extreme response- hypoglycemia)
    - Low hsCRP in patient with clinical diagnosis of T2DM
    - Biochemical/Molecular phenotypic evidence from patient cell lines
    - Hepatocellular adenomas (PMID: 31166087)

BENIGN CRITERIA			
	Rule	Original Description	Specifications
SA	BA1	Allele frequency greater than expected for disorder (standalone):	MAF ≥ 1:10,000 (≥ 0.01% or 0.0001) in gnomAD Popmax Filtering AF.
Strong	BS1	Allele frequency greater than expected for disorder	MAF ≥ 1:30,000 (≥0.0033% or 0.000033) in gnomAD Popmax Filtering AF.
	BS2	Observation in controls inconsistent with disease penetrance	Apply to normoglycemic individuals age 70 or older (i.e., genotype positive, phenotype negative)
	BS3	Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing	Applicable to non-canonical splice site variants that have RNA and in silico evidence of normal splicing.
	BS4	Lack of segregation in affected members of a family	Applicable to family members without variant who have MPC score >50% (i.e., genotype negative, phenotype positive).
Supporting	BP1	Missense variant in a gene for which primarily truncating variants are known to cause disease	Not applicable
	BP2	Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern.	Also applicable when in cis or trans with a likely pathogenic variant.
	BP3	In-frame deletions/insertions in a repetitive region without a known function	Not applicable
	BP4	Multiple lines of computational evidence support a benign effect on the gene or gene product	Use a REVEL score of ≤0.15 as supportive evidence of no predicted impact on the gene or gene product. We also support using SpliceAI to assess the predicted impact of non-canonical splicing variants and synonymous variants: apply BP4 when the predicted change is below 0.2 (Wai et al. 2020 PMID: 32123317; Jaganathan et al. 2019 PMID: 30661751).
	BP5	Variant found in a case with an alternate	A variant in other monogenic diabetes gene is P/LP.

		molecular basis for disease	
	BP6	Reputable source describes as benign	Removed per SVI recommendations
	BP7	A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.	Use with no specifications.
	BS3_Supporting	Gene-specific/ Modified strength	See list of approved functional studies and guidelines for interpretation of data.

Criterion	Fraction	Decimal	Percent	Reference	Rationale
BA1	>=1/10,000	0.0001	0.01%	Popmax Filtering AF	Lowest frequency benign variant p.Arg114His has Popmax Filtering AF = 0.00009 in gnomAD genomes
BS1	>=1/30,000	0.000033	0.0033%	Popmax Filtering AF	Mid-penetrant variant p.Pro467Leu has Popmax Filtering AF= 0.000023 in gnomAD exomes
PM2_Supporting	<=1/50,000	0.00002	0.002%	Non-Finnish Europeans	Non-Finnish European frequency of most common known pathogenic variants (p.Pro447Leu and p.Glu275del) = 0.000018 and (2 copies each)
	And <= 1 copy	<= 1 copy	<= 1 copy	Other founder and non-founder populations	Other populations have small N so finding 1 copy by chance will make it high but should not rule out it being rare

BS3_Supporting:

- Luciferase assays for transactivation
    - "No functional impact" is defined as ≥75% activity of wildtype. If data from more than one experiment is available, do not apply BS3_Supporting if one result is ≥75% and the other is not.
    - Note: this threshold is not 100% specific for transactivation (TA) activity and is complicated by the fact that TA activity will vary depending on many factors, for instance cell line used (HeLa,

INS, MIN6 etc). Please see PS3_Supporting section above for recommendations on controls. The degree of TA impairment of HNF1A tends to correlate positively with severity of phenotype: that is MODY causal HNF1A variants impair TA activity more severely (<40% compared to WT) than T2D risk variants (40-75% compared to WT).

- EMSA for DNA binding
  - "No functional impact" is defined as ≥75% activity of wildtype.
  - Note: the effect of the variant on DNA binding will be highly dependent on whether the variant is located within the DNA binding domain. Please see PS3_Supporting section above for recommendations on controls.
- Western blotting and indirect immunofluorescence for protein expression & localization
  - Determining appropriate thresholds for protein expression is more difficult due to variability in results between different experimental protocols. Sample preparations, gel loading, transfer efficiency, antibody specificity, choice of internal control and quantification method are some of the factors that can contribute to varying and inconsistent results between labs. Altered protein expression can be indirectly captured through the read-out from a transactivation assay and reduced protein expression can provide an explanation for reduced transactivation.To use BS3, functional study must have been performed on a transfected variant. If a study was performed on a cell line generated from a patient sample (and therefore contains the variant plus wild-type allele and other variants in the patient's genome) it cannot count as BS3.
- References
  - Najmi et al (2017) (PMID: 27899486)
  - Bjorkhaug et al (2003) (PMID: 12574234)
  - Brnich et al (2020) (PMID: 31892348)
  - Althari et al (2020) (PMID: 32910913)